Package 'flowAI'

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Type Package

Title Automatic and interactive quality control for flow cytometry data

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Description The package is able to perform an automatic or interactive quality control on FCS data acquired using flow cytometry instruments. By evaluating three different properties: 1) flow rate, 2) signal acquisition, 3) dynamic range, the quality control enables the detection and removal of anomalies.

Depends R (>= 4.3.0)

License GPL (>= 2)

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Imports ggplot2, flowCore, plyr, changepoint, knitr, reshape2, RColorBrewer, scales, methods, graphics, stats, utils, rmarkdown

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`Bcells` *flowSet of B cells.*

Description

This data set contain three flowFrame objects created by subsetting three FCS files of an Aging study made in Singapore. The samples were stained with a panel aimed to identify B cell subpopulations. The data is stored as a flowSet object, a class implemented in the flowCore package to handle FCS files in R.

Usage

```
data(Bcells)
```

Format

A flowSet containing 3 flowFrames

`flow_auto_qc` *Automatic quality control of flow cytometry data.*

Description

For a set of FCS files, *flow_auto_qc* performs a complete and automatic quality control. It consists in the detection and removal of anomalies by checking three properties of flow cytometry: 1) flow rate, 2) signal acquisition, 3) dynamic range.

Usage

```
flow_auto_qc(
  fcsfiles,
  remove_from = "all",
  output = 1,
  timeCh = NULL,
  timestep = NULL,
  second_fractionFR = 0.1,
  alphaFR = 0.01,
  ModeDevFR = NULL,
  decompFR = "cffilter",
```

```
  ChExcludeFS = c("FSC", "SSC"),
  outlier_binsFS = FALSE,
  pen_valueFS = 500,
  max_cptFS = 3,
  ChExcludeFM = c("FSC", "SSC"),
  sideFM = "both",
  neg_valuesFM = 1,
  html_report = "_QC",
  mini_report = "QCmini",
  fcs_QC = "_QC",
  fcs_highQ = FALSE,
  fcs_lowQ = FALSE,
  folder_results = "resultsQC",
  ...
)
```

Arguments

`fcsfiles`	It can be a character vector with the filenames of the FCS files, a flowSet or a flowFrame.
`remove_from`	Select from which of the three steps the anomalies have to be excluded in the high quality FCS file. The default option `"all"` removes the anomalies from all the three steps. Alternatively, you can use: `"FR_FS"`, `"FR_FM"`, `"FS_FM"`, `"FR"`, `"FS"`, `"FM"`, to remove the anomalies only on a subset of the steps where *FR* stands for the flow rate, *FS* stands for signal acquisition and *FM* stands for dynamic range.
`output`	Set it to 1 to return a flowFrame or a flowSet with high quality events only. Set it to 2 to return a flowFrame or a flowSet with an additional parameter where the low quality events have a value higher than 10,000. Set it to 3 to return a list with the IDs of low quality cells. Set it to any other value if no R object has to be returned. Default is `1`.
`timeCh`	Character string corresponding to the name of the Time Channel in the set of FCS files. By default is `NULL` and the name is retrieved automatically.
`timestep`	Numerical value that specifies the time step in seconds. In other words, it tells how many seconds one unit of time corresponds to. By default is `NULL` and the value is retrieved automatically.
`second_fractionFR`	The fraction of a second that is used to split the time channel in order to recreate the flow rate. Set it to `"timestep"` if you wish to recreate the flow rate at the maximum resolution allowed by the flow cytometry instrument. Usually, for FCS files the timestep corresponds to 0.01, however, to shorten the running time of the analysis the fraction used by default is 0.1, corresponding to 1/10 of a second.
`alphaFR`	The level of statistical significance used to accept anomalies detected by the ESD outlier detection method. The default value is `0.01`. Decrease the value to make the flow rate check less sensitive.

`ModeDevFR` If defined, it will remove parts of the flow rate that are a number of standard deviation from the mode of the trend. To perform this filter, add the number to multiply to the standard deviation. It is suggested a number between 1 and 2. Default is NULL, hence not performed.

`decompFR` Default is "cffilter" and it will use the Christiano-Fitzgerald method to calculate the trend and cycle components. Any other value will perform Loess regression to predict the trend line. In this case the cycle component will be the distances from the trend line.

`ChExcludeFS` Character vector with the names or name patterns of the channels that you want to exclude from the signal acquisition check. The default option, `c("FSC", "SSC")`, excludes the scatter parameters. If you want to include all the parameters in the analysis use `NULL`.

`outlier_binsFS` logical indicating whether outlier bins (not events) have to be removed before the changepoint detection of the signal acquisition check. The default is `FALSE`.

`pen_valueFS` The value of the penalty for the changepoint detection algorithm. This can be a numeric value or text giving the formula to use; for instance, you can use the character string `"1.5*log(n)"`, where n indicates the number of cells in the FCS file. The higher the penalty value the less strict is the detection of the anomalies. The default is `500`.

`max_cptFS` The maximum number of changepoints that can be detected for each channel. The default is `3`.

`ChExcludeFM` Character vector with the names or name patterns of the channels that you want to exclude from the signal acquisition check. The default option, `c("FSC", "SSC")`, excludes the scatter parameters. If you want to include all the parameters in the analysis use `NULL`.

`sideFM` Select whether the dynamic range check has to be executed on both limits, the upper limit or the lower limit. Use one of the options: `"both"`, `"upper"`, `"lower"`. The default is `"both"`.

`neg_valuesFM` Scalar indicating the method to use for the removal of the anomalies from the lower limit of the dynamic range. Use `1` to remove negative outliers or use `2` to truncate the negative values to the cut-off indicated in the FCS file.

`html_report` Suffix to be added to the FCS filename to name the HTML report of the quality control. The default is `"_QC"`. If you do not want to generate a report use `FALSE`.

`mini_report` Name for the TXT file containing the percentage of anomalies detected in the set of FCS files analyzed. The default is `"_QCmini"`. If you do not want to generate the mini report use `FALSE`.

`fcs_QC` Suffix to be added for the filename of the new FCS containing a new parameter where the low quality events only have a value higher than 10,000. The default is `"_QC"`. If you do not want to generate the quality controlled FCS file use `FALSE`.

`fcs_highQ` Suffix to be added for the filename of the new FCS containing only the events that passed the quality control. The default is `FALSE` and hence the high quality FCS file is not generated.

`fcs_lowQ` Suffix to be added for the filename of the new FCS containing only the events that did not pass the quality control. The default is `FALSE` and hence the low quality FCS file is not generated.

folder_results — Character string used to name the directory that contains the results. The default is "resultsQC". If you intend to return the results in the working directory use FALSE.

... — additional parameters passed to read.flowSet to provide flexibility over how the FCS files are read in.

Value

A complete quality control is performed on flow cytometry data in FCS format. By default the analysis returns:

1. a flowFrame or flowSet object containing new FCS files with only high quality events

and a directory named *resultsQC* containing:

1. a set of new FCS files with a new parameter to gate out the low quality events a value larger than 10,000 is assigned to them only,

2. a set of HTML reports, one for each FCS file, that include graphs and table indicating where the anomalies were detected,

3. a single TXT file reporting the percentage of events removed in each FCS file.

Author(s)

Gianni Monaco, Chen Hao

Examples

```
## a sample dataset as flowSet object
data(Bcells)

## quality control on a flowFrame object
resQC <- flow_auto_qc(Bcells[[1]], html_report = FALSE, mini_report = FALSE, fcs_QC = FALSE, folder_results = FALSE
```

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flow_iQC *Interactive quality control of Flow Cytometry Data*

Description

The call of the flow_iQC function opens a Shiny application that allows to perfom a complete and interactive quality control of an FCS file. The framework of the interactive quality control is complementary to the automatic one of the flow_auto_qc function. Hence, the anomalies are manually selected from the evaluation of three main properties of flow cytometry: 1) flow rate, 2) signal acquisition, 3) dynamic range.

Usage

```
flow_iQC()
```

Author(s)

Chen Hao, Gianni Monaco

Examples

```
if (interactive()) flowAI::flow_iQC()
```

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